

There are several reasons for stressing the importance of identifying chlamydia and understanding its incidence among the STDs. *C trachomatis* is one of the most important causes of sterility in black Africa.⁴ Female sterility is not only a health problem but also a social handicap in African culture. Furthermore, it has been demonstrated that both ulcerative and non-ulcerative STDs play an important role in facilitating transmission of HIV in Africa.^{5,6} In South African prostitutes in 1991, Plummer *et al*⁵ stressed the importance of the mucosal disruption due to *C trachomatis* in facilitating the HIV transmission. Prostitutes, men frequenting prostitutes and men and women with multiple sex partners are the major groups at risk, but it is certainly important to follow the distribution of STDs also in the general population of countries where such diseases are highly diffused and cause infertility, since prevention and public health are major problems.

These findings emphasise the importance of the introduction of the routine diagnosis of *C trachomatis* for the control of STDs diffusion in developing countries.

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Sensitivity of a commercial polymerase chain reaction for different serovars of *Chlamydia trachomatis* present at low titre in clinical samples

Laboratory detection of *Chlamydia trachomatis* is hampered by the fragile and fastidious nature of *Chlamydia*. Considerable efforts have been employed to find a suitable alternative to culture detection which, although highly specific, is costly, tedious and furthermore may only reveal 85% of all infections, even when optimal transport and culture conditions are realised.¹ Numerous authors have developed polymerase chain reaction (PCR) procedures to amplify a variety of chlamydia genes, but it has become apparent that the most simple and sensitive strategy involves targetting a small portion of the ubiquitous *C trachomatis* plasmid.² A commercialised version of this test has been produced (Amplicor *Chlamydia trachomatis*, Roche Diagnostic Systems, Branchburg, NJ) and shown to be more sensitive than culture in numerous settings.^{1,3} Although it has been reported that all *C trachomatis* serovars may be detected by Amplicor,³ this has not been shown in samples where *C trachomatis* was present at low titre. It has, however, been previously demonstrated that other non-culture, *C trachomatis* detection strategies perform less well on clinical samples with few infectious particles.⁴

We have employed this commercial procedure retrospectively to analyse residual transport medium from 55 randomly-chosen clinical samples that had been found to give few chlamydial inclusions in culture.⁵ They were interspersed with 55 culture-negative specimens and analysed by Amplicor³ and an in-house PCR.⁵ Clinical specimens in 2SP transport medium had to be diluted tenfold in Amplicor specimen transport media prior to PCR.

All 55 culture-positive samples were also positive by PCR as was one of the 55 culture-negative samples. The unique PCR-positive, culture-negative specimen was confirmed positive by the in-house PCR procedure and by direct immunofluorescent staining (MicroTrak, Syva Canada, Kanata, Ontario).

The in-house PCR procedure was employed to type the 56 positive samples⁵ but produced sufficient DNA to type only 51 of them. A third round of PCR using primer 5 (GGAGATCCTTGCGATCCTTG) and primer 4⁵ was necessary to amplify the remaining five samples. All of the different serovars observed in an analysis of 435 *C trachomatis*-positive urogenital specimens⁵ were also present in these 56 samples (see table). This indicated that Amplicor PCR was able to detect all of the common urogenital serovars in clinical specimens even when they were present at low titre. The proportion of serovar F strains was high in this survey as was expected from previous reports that identified this serovar more frequently among isolates with few inclusions in culture.⁵

In the present study a special sample preparation procedure proposed by Roche

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Chlamydia trachomatis serovars in 56 PCR-positive clinical specimens

Number of inclusions per coverslip	Number of samples of serovar									
	B group			Intermediate		C group				
	Bb*	E	D	F	G	Ca*	H	Ia	J	K
0-10	4	13	1	15	3	1	4	2	2	1
10-25	0	2	3	2	0	0	0	0	1	2
Total	4	15	4	17	3	1	4	2	3	3

*urogenital Ba and C isolates are termed Bb and Ca respectively.⁶

Diagnostic Systems was employed in order to use residual sample intended for culture. Despite this tenfold dilution and the deliberate use of clinical specimens with few inclusions in culture, all culture-positive samples were also positive by Amplicor PCR. False-negative PCR results in other studies were possibly the result of inhibitors in the sample. In the present study, the further dilution of the clinical sample has apparently reduced the impact of inhibitors without compromising the sensitivity of the procedure.

Although theoretically one *C trachomatis* elementary body may give rise to an inclusion in culture, it would not be surprising that the elementary body to inclusion-forming unit (IFU) ratio of *C trachomatis* in clinical specimens would be similar to the particle to plaque-forming-unit ratio of mammalian viruses (that is, 100 to 1000) as they both must engineer invasion of an eukaryotic cell and takeover of the cellular machinery while both are subject to inactivation during transport. As there are at least 10 copies of the plasmid per chlamydial elementary body, the ratio of plasmid PCR targets to IFU is then probably superior to 1000. This might explain why a tenfold dilution of the clinical sample did not decrease the sensitivity of Amplicor PCR.

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Urethral flora in adolescent boys

Since the information about the composition of the normal urethral flora in sexually inactive young males is limited, it is difficult to know exactly the significance of various isolates from men with urethritis. After the commencement of sexual activity normal urethral flora is bound to alter, we undertook the study of sexually unexposed individuals. The study was undertaken (1) to ascertain the normal flora of the anterior urethra in 50 adolescent boys (aged 13 to 17 years) before commencement of sexual activity (Group A), (2) to evaluate the flora of 50 recently married men in monogamous relationship with no history of sexually transmitted diseases (STDs) (Group B) and (3) to identify various pathogens based on their isolation in 50 patients with non-gonococcal urethritis (NGU) (Group C) and 50 non-urethritis STD patients (Group D) and comparing them with the isolates from the sexually unexposed adolescents. Use of systemic antibiotics in the preceding two weeks was the criterion for exclusion.

If the secretions were scanty milking of urethra was done prior to the collection of samples after holding urine for at least 4 hours. Gram staining of the urethral smears was done for pus cells and microorganisms, saline smear was prepared for *Trichomonas vaginalis*, Giemsa staining for giant cells suggestive of herpes simplex virus (HSV) infection and 10% KOH smear for detection of yeasts were also carried out.

Urethral swabs were processed for culture of mycoplasmas, *T vaginalis*, aerobes and anaerobes including *Gardnerella vaginalis* and for yeasts. After obtaining the urethral swabs, 10 ml of first voided urine was collected from each subject for smear and culture of *T vaginalis*. For culture, 10 ml of mid-stream urine was collected. 5 ml venous blood was drawn for VDRL and HIV serology (ELISA). Data were analysed by using the chi square test (with or without Yate's correction). Data from Group A were compared separately with data from Groups B, C and D.

All smears were negative for *T vaginalis* and giant cells. KOH smear for yeasts were positive in 2 (4%) NGU cases. None of the urine specimens on culture contained more than 10⁴ bacteria/ml, the cut off count for significance. VDRL test and HIV serology (ELISA) was negative in all subjects. Different organisms isolated are shown in the table.

Sexually unexposed adolescents had predominantly the aerobic flora which mostly included the resident cutaneous flora viz